Appl. No.

10/630,629

**Filed** 

July 29, 2003

## AMENDMENTS TO THE SPECIFICATION

On page 9, please replace the paragraph between lines 6 and 8 with the following paragraph:

Nucleotide and deduced amino acid sequence alignment of partial ORF of *eba*-175 (*ebp1*) (SEQ ID NO: 18), *ebp2* (SEQ ID NO: 1), *ebp3* (SEQ ID NO: 2), *ebp4* (SEQ ID NO: 3) and *ebp5* (SEQ ID NO: 4). Identical amino acid residues are shown in boxes.

On page 33, please replace the paragraph starting at line 23 and ending on page 34, line 5, with the following paragraph:

Aliquots of purified mRNA, isolated from purified schizont infected erythrocytes using a mRNA isolation kit (Stratagene, La Jolla, CA), were stored precipitated in ethanol with 3M sodium acetate at -70°C. The mRNA was treated with DNAase to ensure that it was free of genomic DNA; the absence of DNA was confirmed by the lack of amplification in RT-PCR studies in the absence of reverse transcriptase. First strand cDNA transcripts were prepared using a poly dT primer from a cDNA CYCLETM kit (Invitrogen, Carlsbad, CA). This first strand oligonucleotide forward amplified by PCR using the primer product was 3' (SEQ ID NO: 6) 5'CAAGGAGAATGTATGGAAAGTA and reverse primer 5'ATCTTCATATTCATTTGGACTCT 3' (SEQ ID NO: 7). The PCR amplified product was detected by ethidium bromide staining a 1% agarose gel.

On page 34, please replace the paragraph starting at line 9 and ending on page 25, line 17, with the following paragraph:

P. falciparum EBP2 RII (amino acids 147-762, 1848bp) was amplified using AdvanTaq Plus™ DNA polymerase (Clontech, Palo Alto, CA) from 100 ng of 3D7 genomic DNA using the forward primer 5'ATGCGGATCCCAATATACGTTTATACAGAAACGTACTC 3' (SEQ ID NO: 8) and reverse primer 5'ATGCGGATCCTCATATATCGTGTTTTTGTTTTAGG 3' (SEQ ID NO: 9) which both contained a BamHI site and the reverse primer contained an additional internal stop codon for cloning into the shuttle vector PCR-Script™ as described by the manufacturer's instructions (Stratagene, La Jolla, CA). The ebp2 RII gene fragment excised with BamHI and cloned into the expression plasmid vector VR1020 (identified as pEBP2-RII). The VR1020 plasmid vector utilizes the human cytomegalovirus promoter and intron A, and human tissue plasminogen activator as the secretory signal and the bovine growth hormone

Appl. No. : 10/630,629 Filed : July 29, 2003

transcriptional terminator/polyadenylation signal (Hartikka et al., 1996). A clone was selected for correct orientation by restriction-enzyme mapping. Both the forward and reverse strands of the ebp2 RII ORF were sequenced using primers off of the vector and primers based on the cloned sequences (Veritas, Inc., Rockville, MD). Human melanoma cells (UM449), were transiently transfected with the plasmids pEBP2RII, 3D7 encoded pEBA-175RII and VR1020 plasmid with Lipofectamine<sup>TM</sup> following the manufacture's protocol (Life Technologies, Gaithersburg, MD). Secretion of RII protein in culture supernatants was confirmed by Western blot. Plasmids were prepared for immunizations using an EndoFree Plasmid Giga kit (Qiagen, Valencia, CA). Purity was gauged by UV spectroscopy (260 nm/280 nm was between 1.70 and 1.90), agarose gel electrophoresis showing predominately supercoiled plasmid and endotoxin levels (<10 EU/mg) were detected using the Limulus amebocyte assay. The ebp2 RVI was amplified by PCR as above except used Vent DNA polymerase (New England BioLabs, Beverly, MA) using the forward primer 5'TCTAGAGATACTAAAAGAGTAAGG 3' (SEQ ID NO: 10) and reverse primer 5'TGATTGACCCTCGCTTTTAAAAC 3' (SEQ ID NO: 11). The PCR amplified fragment was gel purified and both the forward and reverse strands were sequenced directly (Veritas, Inc.).

## On page 42, please replace the paragraph starting at line 27 and ending on page 43, line 14, with the following paragraph:

DNA vaccine that encoded region II of EBP3 was constructed similarly to EBP2 region. II. Forward primer for EBP3: 5' ATGC GGA TCC GAA AAG AAT AAA TTT ATT GAC ACT 3' BamHI (SEQ ID NO: 12); Reverse primer for EBP3: 5'ATGC GGA TCC TCA AGG AAA CAC ATT CGT TTT TAT AGG 3' BamHI (SEQ ID NO: 13). Mice were immunized and polyclonal immune sera were tested for recognition of parasite proteins by IFAT on methanol fixed parasitized erythrocytes. The results are shown in the Table 1 above. The IFAT results were all negative for detection of an expressed parasite protein except for the novel protein EBP2. EBA-175 was used as a positive control in these studies. EBP3 polyclonal immune was also tested by immunoblot against supernatant collected from VM92 cells transiently transfected individually with the EBP DNA vaccine. The results were all negative except for EBP2 for self-recognition of a transiently expressed region II fragment of EBP2. Although the results for EBP3 was negative here, these studies do not exclude the expression of this protein in erythrocytic

Appl. No. : 10/630,629 Filed : July 29, 2003

stage parasites or in other parasite stages e.g., sporozoite, exo-erythrocytic, or sexual stage parasites.

## On page 43, please replace the paragraph starting at line 19 and ending on page 44, line 10, with the following paragraph:

DNA vaccine that encoded region II of EBP4 was constructed by directly cloning EBP4 into the DNA vaccine VR1020. Specific forward and reverse primers with BamHI restriction sites were used to PCR amplify from genomic (P. falciparum) DNA (Fig. 2) using standard molecular biological techniques that are known in the art. Forward primer for EBP4: 5'ATGC GGA TCC AAT CTG AAA GCT CCA AAT GCT AAA TCC 3'BamHI (SEQ ID NO: 14); Reverse primer for EBP4: 5'ATGC GGA TCC TCA TAT AGG AAA CAC ATT CGT TTT TAT AGG 3'BamHI (SEQ ID NO: 15). Mice were immunized and polyclonal immune sera were tested for recognition of parasite proteins by IFAT on methanol fixed parasitized erythrocytes. The results are shown in the Table 1 above. The IFAT results were all negative for detection of an expressed parasite protein except for the novel protein EBP2. EBA-175 was used as a positive control in these studies. EBP4 polyclonal immune was also tested by immunoblot against supernatant collected from VM92 cells transiently transfected individually with the EBP DNA vaccine. The results were all negative except for EBP2 for self-recognition of a transiently expressed region II fragment of EBP4. Although the results for EBP4 was negative here, these studies do not exclude the expression of this protein in erythrocytic stage parasites or in other parasite stages such as sporozoite, exo-erythrocytic, or sexual stage parasites.

## On page 44, please replace the paragraph starting at line 15 and ending on page 45, line 6, with the following paragraph:

DNA vaccine that encoded region II of EBP5 was constructed by directly cloning EBP5 into the DNA vaccine VR1020. Specific forward and reverse primers with BamHI restriction sites were used to PCR amplify from genomic (*P. falciparum*) DNA (Fig. 2) using standard molecular biological techniques that are known in the art. Forward primer for EBP5: 5'ATGC GGA TCC AAT AGA AAT AGT TTT GTT CAA 3'BamHI (SEQ ID NO: 16); Reverse primer for EBP5: 5'ATGC GGA TCC TCA TGA GTC TAT AGA TAA CAT TTC 3'BamHI (SEQ ID NO: 17). Mice were immunized and polyclonal immune sera were tested for recognition of parasite proteins by IFAT on methanol fixed parasitized erythrocytes. The results are shown in

Appl. No. : 10/630,629 Filed : July 29, 2003

the Table 1 above. The IFAT results were all negative for detection of an expressed parasite protein except for the novel protein EBP2. EBA-175 was used as a positive control in these studies. EBP5 polyclonal immune was also tested by immunoblot against supernatant collected from VM92 cells transiently transfected individually with the EBP DNA vaccine. The results were all negative except for EBP2 for self-recognition of a transiently expressed region II fragment of EBP2. Although the results for EBP5 was negative here, these studies do not exclude the expression of this protein in erythrocytic stage parasites or in other parasite stages such as sporozoite, exo-erythrocytic, or sexual stage parasites.